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Proposed Registration Decision PRD2012-32

# *Bacillus subtilis* var. amyloliquefaciens Strain FZB24

*(publié aussi en français)*

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# Overview

## Proposed Registration Decision for Taegro Technical

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of Taegro Technical and Taegro, containing the technical grade active ingredient *Bacillus subtilis* var. *amyloliquefaciens* Strain FZB24, to suppress fusarium wilt on greenhouse cyclamen, fusarium head blight on wheat; bottom rot on lettuce and late blight on tomato.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of Taegro Technical and Taegro.

## What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on Taegro Technical, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on Taegro Technical, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

## What Is Taegro Technical?

Taegro Technical contains the active ingredient *Bacillus subtilis* var. *amyloliquefaciens* strain FZB, which is a bacterium that is used as a microbial pest control agent (MPCA) to suppress soil-borne diseases by *Fusarium* spp., *Rhizoctonia* spp. and *Phytophthora* spp. *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 acts as a plant growth promoter and also produces various antifungal agents and enzymes. Strain FZB24 of *B. subtilis* var. *amyloliqufaciens* was originally isolated from soil in Germany.

The new end-use product, Taegro, contains *B. subtilis* var. *amyloliquefaciens* strain FZB24 as the active ingredient. Taegro is proposed for use as commercial-class biological fungicide to suppress bottom rot on lettuce (greenhouse and field), late blight on tomato (greenhouse and field), *Fusarium* wilt on greenhouse cyclamen and *Fusarium* head blight on wheat.

## Health Considerations

### Can Approved Uses of *B. subtilis* var. *amyloliquefaciens* strain FZB24 Affect Human Health?

***Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is unlikely to affect your health when Taegro is used according to the label directions.**

People could be exposed to *B. subtilis* var. *amyloliquefaciens* strain FZB24 when handling and applying Taegro. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When spores of *B. subtilis* var. *amyloliquefaciens* strain FZB24 were tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

**Residues in Water and Food**

**Dietary risks from food and water are not of concern**

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure that the food Canadians eat is safe.

*Bacillus subtilis* var. *amyloliquefaciens,* the active ingredient in Taegro Technical and Taegro, is a ubiquitous bacterium that is commonly found in soil. When *B. subtilis* var. *amyloliquefaciens* strain FZB24 was administered orally to rats, no signs of toxicity or disease were observed, and no metabolites of toxicological significance have been shown to be produced by this strain of *B. subtilis* var. *amyloliquefaciens*. Although some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning, these strains demonstrated the ability to produce a highly heat-stable toxin that may be similar to a toxin produced by *Bacillus cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 2000. Therefore the establishment of a MRL is not required for *B. subtilis* var. *amyloliquefaciens* strain FZB24. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

**Occupational Risks From Handling Taegro**

**Occupational risks are not of concern when Taegro is used according to label directions, which include protective measures**

Workers handling Taegro can come into direct contact with *B. subtilis* var. *amyloliquefaciens* strain FZB24 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers exposed to the end-use product must wear waterproof gloves, long-sleeved shirts, long pants, goggles, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks.

For the bystander, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

## Environmental Considerations

### What Happens When Taegro Is Introduced Into the Environment?

#### Environmental risks are not of concern

Information on the environmental fate of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 suggests that, as a soil microorganism, it is likely that *B. subtilis* var. *amyloliquefaciens* strain FZB24 could survive in soil at elevated levels under suitable environmental conditions (i.e. type of soil, moisture, acidity levels, and temperature). However, the populations of *B. subtilis* var. *amyloliquefaciens* strain FZB24 should return to naturally occurring levels over time.

Studies were conducted to determine the effects of *B. subtilis* var. *amyloliquefaciens* strain FZB24 on birds, fish, bees, aquatic arthropods, terrestrial non-arthropod invertebrates and algae. These studies showed that *B. subtilis* var. *amyloliquefaciens* strain FZB24 was not toxic or pathogenic to birds, fish, bees, aquatic arthropods, and terrestrial non-arthropod invertebrates. Adverse effects to the growth of algae were observed, however, these observations were attributed to physical properties of the bacterial suspension rather than toxicity, i.e. test suspensions were opaque which reduced photosynthesis.

In published literature, other strains of *B. subtilis* have been reported to cause infections in mammals, terrestrial insects and plants; however, these reports were few in number considering the large amount of published literature on this microorganism. Furthermore, these reports involved unusual strains, or select strains, of *B. subtilis* for which their ability to cause disease was not thoroughly investigated. There are no reports with *B. subtilis* var. *amyloliquefaciens* strain FZB24 in non-target organisms except for the intended pest.

## Value Considerations

### What Is the Value of Taegro?

Taegro is a non-conventional fungicide that provides suppression of tomato late blight and lettuce bottom rot as well as partial suppression of fusarium wilt on cyclamen and fusarium head blight on wheat. Taegro represents an alternative mode of action to conventional fungicides and poses a low risk of resistance development.

## Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Taegro to address the potential risks identified in this assessment are as follows.

**Key Risk-Reduction Measures**

**Human Health**

Individuals exposed to large quantities of Taegro could possibly, upon repeated exposure to the product, develop respiratory and dermal sensitivity since Taegro has been identified as a sensitizer. Therefore, anyone handling or applying Taegro must wear waterproof gloves, long-sleeved shirts, long pants, a NIOSH-approved respirator (with any N-95, P-95, R-95 or HE filter for biological products), and shoes plus socks. Due to the irritation potential identified for Taegro, workers and handlers are also required to wear eye goggles. Also, the signal words, "POTENTIAL SENSITIZER" and "WARNING-EYE and SKIN IRRITANT" on the principal display panel and precautionary statements, "DO NOT get in eyes or on skin" and "May cause sensitization"are required on the secondary display panel of the label.

**Environment**

The end-use product (Taegro) label will include environmental precaution statements that prevent the contamination of aquatic systems from it use.

## Next Steps

Before making a final registration decision on *Bacillus subtilis* var. *amyloliquefaciens* Strain FZB24, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

## Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Bacillus subtilis* var. *amyloliquefaciens* Strain FZB24 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

# Science Evaluation

## 1.0 The Active Substance, its Properties and Uses

### 1.1 Identity of the Active Ingredient

| | |
|---|---|
| **Active microorganism** | *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 |
| **Function** | To suppress bottom rot on lettuce (greenhouse and field), late blight on tomato (greenhouse and field), *Fusarium* wilt on greenhouse cyclamen and *Fusarium* head blight on wheat. |
| **Binomial name** | *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 |
| **Taxonomic designation**[1] | |
| **Kingdom** | Eubacteria |
| **Phylum** | Firmicuties |
| **Class** | Bacilli |
| **Order** | Bacillales |
| **Family** | Bacillaceae |
| **Genus** | *Bacillus* |
| **Species Group** | *subtilis* group |
| **Species** | *amyloliquefaciens* |
| **Strain** | FZB24 |
| **Patent Status information** | No patents are held by the applicant in Canada. |
| **Minimum purity of active** | Technical grade active ingredient (TGAI): $2.0 \times 10^{11}$ colony forming units (CFU)/g<br>End-use product (EP): $1.0 \times 10^{10}$ CFU/g |
| **Identity of relevant impurities of toxicological, environmental and/or significance.** | The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Bacillus subtilis* var. *amyloliquefciens* strain FZB24 is not known to produce any toxic secondary metabolites (see Section 3.0). |

[1] Taxonomy browser at: http://www.ncbi.nlm.nih.gov/pubmed/

### 1.2 Physical and Chemical Properties of the Technical Grade Active Ingredient and the End-use Products

**Technical Grade Active Ingredient–Taegro Technical and End-use Product–Taegro**

| Properties | Taegro Technical | Taegro |
|---|---|---|
| Physical state | Solid | Solid |
| Colour | Beige | Beige |
| Odour | Acidic | Carbonyl |
| pH (1% w/v) | 6.9 | 6.6 |
| Bulk Density | 0.62 g/mL | 0.718 g/mL |
| Guarantee | $2.0 \times 10^{11}$ CFU/g; 79.9% w/w | $1.0 \times 10^{10}$ CFU/g; 13.0% w/w |
| Corrosion Character | None | None |
| Moisture Content | 4.4–10.3% | 7.7–9.5% |

### 1.3 Directions for Use

Depending on the targeted crop, Taegro is to be applied preventatively as a foliar spray, a drench application or a spray directed to the plant base. Applications should be made at 7-day intervals when conditions are conducive to disease development.

### 1.4 Mode of Action

*B. subtilis* var. *amyloliquefaciens* strain FZB24 is reported to antagonize fungal pathogens through antibiosis and induction of host plant resistance.

## 2.0 Methods of Analysis

### 2.1 Methods for Identification of the Microorganisms

*Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 can be identified to the species level using a combination of colony morphologies on agar media and polymerase chain reaction (PCR) to amplify 16S rRNA gene. *B. subtilis* var. *amyloliquefaciens* Strain FZB24 has two different morphologies on agar media, i.e. a dominant morphology and a secondary morphology, which can help distinguish this strain of *B. subtilis* var. *amyloliquefaciens* from other naturally occurring strains. PCR amplification of the 16 rRNA gene is a valid method for identifying microorganisms to the species level.

## 2.2 Methods for Establishment of Purity of Seed Stock

Stock cultures of *B. subtilis* var. *amyloliquefaciens* strain FZB24 are maintained in the German Strain Collection for Microorganisms (DSMZ) as well as the United States Agricultural Research Service – Culture and Patent Culture Collections. Stock solutions are kept frozen at -80°C.

Practices for ensuring the purity of the seed stock were adequately described in the method of manufacture and quality assurance program.

## 2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The potency (CFU/g) of the technical grade active ingredient and the end-use products will be determined by plate counting on agar media.

## 2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As noted in Section 2.1, the microbial pest control agent (MPCA) can be identified to the species level using a combination of colony morphologies on agar media and PCR to amplify the 16S rRNA gene. These methods can help to identify *B. subtilis* var. *amyloliquefaciens* strain FZB24, however, these methods alone may not be sufficient to distinguish this strain of *B. subtilis* var. *amyloliquefaciens* from all other naturally occurring strains. No methods are required to quantify viable or non-viable residues of *B. subtilis* var. *amyloliquefaciens* strain FZB24. *Bacillus subtilis* var. *amyloliquefaciens* is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments. According to the United States Food and Drug Administration, some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat-stable toxin that may be similar to the vomiting type toxin produced by *Bacillus cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 2000. Furthermore, when *B. subtilis* var. *amyloliquefaciens* strain FZB24 was administered orally to rats, no signs of toxicity or disease were observed.

## 2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of Taegro Technical and Taegro are acceptable.

During manufacturing, several approaches will be used to limit microbial contamination in the Technical grade active ingredients and end-use product. These approaches will include purity checks using microscopic techniques, and plating on selective agar media, sterilization of all equipment and media, and sanitization of recovery equipment.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of five production batches using microbe-specific screening methods for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants comply with those permitted by the PMRA and are adequate to ensure that the end-use product does not contain unacceptable levels of human and animal disease-causing microorganisms.

No known toxic metabolites or hazardous substances are present in Taegro.

### 2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Results from storage stability testing of two batches of the technical grade active ingredient and one batch of the end-use product showed that these products are stable when stored at 4–35°C for a period of up to 1 year.

## 3.0 Impact on Human and Animal Health

### 3.1 Toxicity and Infectivity Summary

A survey of published literature has revealed a number of instances where other *B. subtilis* strains had been implicated in infections in humans as well as the causal agent in food poisonings. Postoperative cellulitis, septicemia, respiratory disease, endocarditis and pneumonia have been reported in humans. In many instances, the association of *B. subtilis* is not sufficiently rigid for it to be regarded unequivocally as the causative agent. Also, the number of putative infections is extremely low considering the total number of reports of bacterial infections. Many of those cases involved drug abuses or severely debilitated patients. As *B. subtilis* is ubiquitous in the environment, it is expected that *B. subtilis* may sometimes be found in association with other microorganisms in infections. Only individuals treated with immunosuppressive drugs appear to be susceptible to infection from *B. subtilis*. In food-borne illnesses, the United States Food and Drug Administration noted that some strains of *B. subtilis* have been isolated from food implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat-stable toxin that may be similar to the vomiting type toxin produced by *B. cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is not reported to produce this toxin. No such illnesses were reported for this microorganism in the United States where it has been registered for use on crops since 2000.

In other mammals, *B. subtilis* has been implicated in cases of bovine mastitis and reproductive disorders in goats. In the cases of bovine mastitis, *B. subtilis* could not be excluded as the causative agent. In goats exhibiting reproductive problems, high bacterial loads in infected vaginas were found to correlate with clinical symptoms. However, *B. subtilis* isolated from infected tissue was not pathogenic to Swiss white mice.

*Bacillus subtilis* has been reported to produce small antibiotic peptides and peptidolipids that are predominantly active against Gram-positive bacteria but also against Gram-negative bacteria, yeast and fungi. These include intracellular peptidolipids (mycosubtilin), extracellular cyclic peptidolipids (*Aspergillus* factor, bacillomycin A/B/D/F/L/R/S, eumycin, fengycin, iturin A and toximycin) and extracellular cyclic peptides (chaetomacin, fungistatin, mycobacillin and *Rhizoctonia* factor). Hemolytic activity has been reported for some peptidolipids.

A detailed review of the toxicological database for *B. subtilis* var. *amyloliquefaciens* strain FZB24 has been completed. The database for *B. subtilis* var. *amyloliquefaciens* strain FZB24 is complete (see Appendix I) consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, acute intravenous, and acute dermal toxicity/irritation studies) currently required for health hazard assessment purposes which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. A waiver request for hypersensitivity incidence testing data was deemed acceptable as PMRA considers all MPCAs to have sensitization potential. As there is adequate information available to characterize the irritation potential of the end-use product, no studies on dermal and eye irritation with the end-use product were required.

The scientific quality of the data is high and the database is considered sufficiently complete to characterize the toxicity and infectivity of this MPCA and the end-use product.

In the acute oral toxicity/pathogenicity study, no significant toxicity was observed in CD rats following oral gavage with $1.3 \times 10^8$ CFU (colony forming units)/animal of *B. subtilis* var. *amyloliquefaciens* strain FZB24. The test substance was cleared from all tissues by Day 7. Based on the results of this study, *B. subtilis* var. *amyloliquefaciens* strain FZB24 is of low toxicity in the rat when challenged via the oral route.

In the pulmonary toxicity/pathogenicity study, no significant toxicity was observed in CD rats following intratracheal administration of 0.1 mL volume suspension of *B. subtilis* var. *amyloliquefaciens* strain FZB24, equivalent to at least $1.4 \times 10^8$ viable CFU/animal. There were no mortalities or treatment-related clinical signs of toxicity. The test substance was detected in the lungs and associated lymph nodes of male and female treatment-group animals on all days tested (Days 0, 7, 21 and 35) and in other tissues, such as liver, kidney and spleen. The test substance was cleared by Day 21 from all tissues tested except from the lungs and associated lymph nodes. Although total clearance was not achieved, a definitive pattern of clearance from the kidneys, liver, and spleen was established. Based on the results of this study, *B. subtilis* var. *amyloliquefaciens* strain FZB24 is of low toxicity and is not infective or pathogenic by the pulmonary route in the rat.

In the intravenous infectivity study, no mortalities or treatment-related clinical signs of toxicity were observed in CD rats following injection with a single dose of 0.5 mL volume suspension of *B. subtilis* var. *amyloliquefaciens* strain FZB24, equivalent to at least $1.0 \times 10^7$ CFU/animal. The test substance was cleared by Day 35 from all tissues except for detection in the spleen of female treatment-group animals. There were no abnormal necropsy findings. Based on the results of this study, *B. subtilis* var. *amyloliquefaciens* strain FZB24 is of low toxicity and is not infective or pathogenic by the intravenous route in the rat.

In the acute dermal toxicity study, no mortalities or treatment-related clinical signs of toxicity other than severe irritation was observed in rabbits exposed for 24 hours to *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient; $3.7 \times 10^{12}$ CFU/g) at a dose of 2000 mg/kg body weight (bw; equivalent to $1.5–2 \times 10^{12}$ CFU/animal) to an area of approximately 10% of body surface area. All animals exhibited erythema, edema, and eschar formation at the application sites during the study. Severe erythema was observed in all animals on Days 3-7 and very slight to severe edema was observed in four animals on Day 3. By Day 11, all animals had recovered from signs of dermal irritation. Based on the results of this study, *B. subtilis* var. *amyloliquefaciens* strain, FZB24 is of low toxicity by the dermal route in rabbit and is considered to be severely irritating to rabbit skin.

In the primary dermal irritation study, no signs of dermal irritation were observed in rabbits dermally exposed for 4 hours to a single dose of 0.5 g of the granular test substance ($7.0 \times 10^{10}$ CFU/g) and a single dose of 0.5 mL volume of 1.5% w/v suspension of *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) separately on two different sites (each test site: $6cm^2$) on one side of the clipped dorsal region of each animal under occlusion. A dermal irritation study was not required by the PMRA for the technical grade active ingredient.

In the acute dermal toxicity study with the end-use product, no mortalities or treatment-related clinical signs of toxicity other than irritation was observed in rabbits (5/sex) exposed for 24 hours to the end-use product at a dose of 2000 mg/kg bw to an area of approximately 10% of body surface area (*B. subtilis* var. *amyloliquefaciens* strain FZB24; $1.7–2.3 \times 10^{11}$ CFU/animal) under occlusion. Erythema, edema, and eschar formation were observed in the study. By Day 8, all animals were free of dermal irritation. Based on the results of this study, the end-use product is of low toxicity by the dermal route in rabbit and is considered to be severely irritating to rabbit skin.

For these products, both the technical grade active ingredient and the end-use product are to be classified as moderately irritating to the skin despite the lack of irritation noted in the dermal irritation study with the technical grade active ingredient. The rationale for this apparent departure from standard practice of relying on results from dermal irritation study is due to the severe irritation noted in the dermal toxicity studies (24-h exposure) with the technical grade active ingredient and the end-use product. Therefore, both the technical grade active ingredient and end-use product labels are required to have signal words "WARNING-SKIN IRRITANT" on the principal display panel to warn users of the potential for irritation from direct exposure.

An eye irritation study was not required for the technical grade active ingredient or the end-use product. Published information indicates that *B. subtilis* var. *amyloliqufaciens* strain FZB24 is irritating to the eye in the rabbit and is classified by the USEPA as toxicity category II for eye irritation. Based on this information, *B. subtilis* var. *amyloliquefaciens* strain FZB24 is classified as moderately irritating to the eyes, and the principal display panels of both the technical grade active ingredient and end-use product labels are required to have signal words, "WARNING-EYE IRRITANT" to warn users of the potential for eye irritation from direct exposure.

A request to waive the requirement for hypersensitivity incidence testing was submitted based on the rationale that there were no incidents of adverse effects among researchers or employees involved in the manufacture, handling or application of Taegro Technical or Taegro. However, the provided information on the absence of incidents of adverse effects does not guarantee that this product will not elicit hypersensitivity effects in the general population. The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions; therefore the signal words "POTENTIAL SENSITIZER" will be required on the principal display of the technical grade active ingredient and end-use product labels. These labels should also have the precautionary statement "May cause sensitization".

Higher-tier subchronic and chronic toxicity studies were not required based on the toxicity profile demonstrated in the test animals in the Tier I acute oral, pulmonary, toxicity/infectivity and intravenous injection infectivity studies.

The active ingredient, *B. subtilis* var. *amyloliquefaciens* strain FZB24, is not known to be a human pathogen nor an endocrine disruptor. Within the available scientific literature, there are no reports that suggest *B. subtilis* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *B. subtilis* var. *amyloliquefaciens* strain FZB24.

## 3.2 Occupational / Bystander Exposure and Risk Assessment

### 3.2.1 Occupational

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal and/or inhalation. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if; the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus subtilis* has not been identified as a dermal wound pathogen, and there is no indication that it could penetrate intact skin of healthy individuals or produce any toxic secondary metabolite that could be dermally absorbed. Furthermore, dermal toxicity studies in animals demonstrated no signs of systemic toxicity to *B. subtilis* var. *amyloliquefaciens* strain FZB24 and Taegro.

The toxicity testing with the *B. subtilis* var. *amyloliquefaciens* strain FZB24 showed no toxicity or infectivity via the oral, dermal, or pulmonary routes of exposure. The submitted dermal toxicity and irritation study with the end-use product demonstrated a potential for skin irritation, and published information indicates that *B. subtilis* var. *amyloliquefaciens* strain FZB24 is irritating to eye; therefore, precautionary label statements to avoid contact of the end-use product with skin and eyes, and use of proper clothing and personal protection equipment (PPE), such as waterproof gloves and protective eye-wear are required to mitigate occupational exposure concerns.

Although dermal toxicity or toxicity from inhalation exposure is considered minimal from the proposed end-use product use, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, goggles, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Taegro, and no significant occupational risks are anticipated for this product.

### 3.2.2 Bystander

Overall, the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the MPCA and the assumption that precautionary label statements will be followed by commercial applicators in the use of Taegro.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible

## 3.3 Incident Reports Related to Human and Animal Health

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.healthcanada.gc.ca/pesticideincident. Incidents from Canada and the Unites States were searched and reviewed for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24.

As of September 14, 2012, there have been no incidents related to health or the environment reported to the PMRA, nor summarized by the USEPA or the California Department of Pesticide Regulation (CDPR), for products containing *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24.

## 3.4 Dietary Exposure and Risk Assessment

### 3.4.1 Food

The proposed use of Taegro as a drench to seedlings or to newly rooted cuttings or as foliar spray to crops is not expected to result in any dietary exposure concern from *B. subtilis* var. *amyloliquefaciens* strain FZB24 because bacteria, such as *B. subtilis* are ubiquitous organisms found in most terrestrial environments.

Although the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children, or animals because *B. subtilis* var. *amyloliquefaciens* strain FZB24 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity study. Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary and subcutaneous injection toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

### 3.4.2 Drinking Water

The likelihood of *B. subtilis* var. *amyloliquefaciens* strain FZB24 entering neighbouring aquatic environments or surface water run-off from greenhouse or field use of Taegro is considered very low.

No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The end-use product label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested not to allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water. Therefore, potential exposure to *B. subtilis* var. *amyloliquefaciens* strain FZB24 in surface and drinking water is negligible.

### 3.4.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the Agency concludes that *B. subtilis* var. *amyloliquefaciens* strain FZB24 is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCAs (including neurological effects from pre- or post-natal exposures), and cumulative effects on infants and children of the MPCAs and other registered micro-organisms that have a common mechanism of toxicity, does not apply to these MPCAs. As a result, the Agency has not

used a margin of exposure (safety) approach to assess the risks of *B. subtilis* var. *amyloliquefaciens* strain FZB24 to human health.

### 3.5 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a MRL under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

*Bacillus subtilis* are ubiquitous organisms found in most terrestrial environments. Residues of *B. subtilis* var. *amyloliquefaciens* strain FZB24 on treated food crops, at the time of harvest, are also anticipated as the active ingredient is comprised of endospores, which are much more persistent in the environment than vegetative cells. Consequently, the Agency has applied a hazard-based approach for determining whether an MRL is required for this microorganism. Based on the lack of toxicity and pathogenicity effects observed in the acute toxicity and infectivity studies (particularly the oral study) and the fact that *B. subtilis* var. *amyloliquefaciens* strain FZB24 is not known to produce any mammalian toxins, the risks anticipated for dietary exposure are considered low. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that an MRL does not need to be established for *B. subtilis* var *amyloliquefaciens* strain FZB24.

*Bacillus subtilis* var *amyloliquefaciens* strain FZB24 is exempt from the requirement of a food tolerance in the United States.

### 3.6 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the Agency's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. subtilis* var. *amyloliquefaciens* strain FZB24 to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be very low since the product is to be used in agricultural sites and is not allowed for use on turf, residential or recreational areas. Furthermore, few adverse effects from exposure to *B. subtilis* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Taegro, there should not be any increase in potential human health risk.

### 3.7 Cumulative Effects

The Agency has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *B. subtilis* var. *amyloliquefaciens* in the environment, the Agency is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *B. subtilis* var. *amyloliquefaciens* strain FZB24. No cumulative effects are anticipated if the residues of *B. subtilis* var. *amyloliquefaciens* strain FZB24 interact with related strains of this microbial species.

## 4.0 Impact on the Environment

### 4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *B. subtilis* var. *amyloliquefaciens* strain FZB24. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in nontarget organisms are noted in Tier I testing.

*Bacillus subtilis* is a rod-shaped, Gram positive, aerobic, motile (peritichous flagella) bacterium that is ubiquitous in nature. *Bacillus subtilis* is commonly found in soil and in plant litter where they play an important role in the biological cycling of carbon and nitrogen. *Bacillus subtilis* is also found in various other habitats such as fresh water, polluted seawater, deep-sea sediments, foods, milk, pharmaceuticals, etc., usually as a result of environmental contamination (i.e. from soil, dust or colonized plant materials). The primary habitat of *B. subtilis* (including variety *amyloliquefaciens*) is considered to be soil. This species commonly produces proteases and other enzymes that breakdown various macromolecules for growth. Under adverse environmental conditions, *B. subtilis* produces an endospore that allows it to endure extreme conditions of heat and desiccation.

From the proposed use of Taegro , the expected environmental concentration (EEC) following direct application to water is $4.55 \times 10^5$ CFU/L based on the maximum label rate of 364g/ha.

### 4.2 Effects on Non-Target Species

The PMRA has a four-level tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration (MCC) of the MPCA. The MCC is generally derived from the amount of the MPCA or its toxin expected to be available following application at the maximum recommended label rate multiplied by some safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies, i.e. life cycle studies, as well as definitive toxicity testing, for example, $LC_{50}$, $LD_{50}$. Tier

IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms. The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

### 4.2.1 Effects on Terrestrial Organisms

Several studies were submitted to address the hazards of the MPCA to terrestrial non-target organisms. These studies included non-target avian species, terrestrial arthropods and non-arthropod invertebrates.

The acute oral pathogenicity and toxicity study of *B.* subtilis var. *amyloliquefaciens* strain FZB24 to Northern bobwhite quail (*Colinus virginianus*) was assessed over 30 days in accordance with USEPA OCSPP Microbial Pesticide Test Guideline 885.4050, *Avian Oral, Tier I. Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) was administered to a group of birds (30 birds) by oral gavage at a dose of 10 mL/kg body weight [bw] (equivalent to $1 \times 10^{12}$ CFU/kg bw) for 5 consecutive days. Other groups (10 birds/group) were similarly treated with inactivated technical grade active ingredient and sterile water at doses of 10 mL/kg bw, and served as attenuated and negative controls. The 30-day acute oral $LD_{50}$ was greater than 10 mL/kg bw or $1 \times 10^{12}$ CFU/kg bw for five consecutive days and the no observed effect level (NOEL) was 10 mL/kg bw or $1 \times 10^{12}$ CFU/kg bw for five consecutive days. There were no treatment related effects observed. There were no signs of pathogenicity or infectivity. This toxicity and pathogenicity study is classified as acceptable and satisfies the guideline requirement for an avian oral toxicity and pathogenicity study.

In a 29-day dietary toxicity/pathogenicity study, adult honeybees (*Apis mellifera*; 170 bees) were fed *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) in a 30% w/v sucrose and distilled water suspension at a rate of $1\times10^5$ CFU/bee in accordance with USEPA OCSPP Microbial Pesticide Test Guideline 885.4380, *Honeybee Testing, Tier I*. Another group (175 bees) remained untreated and served as a negative control. The study was terminated on Day 29 after all the bees in the study were dead. Mortality was not significantly different between the groups and there were no toxic symptoms observed in the test group during the test period. This toxicity and pathogenicity study is classified as acceptable and satisfies the guideline requirement for a terrestrial arthropod study.

In a 20-day dietary toxicity/pathogenicity study, larval honeybees (*Apis mellifera*; 2–3-day old; 80 larvae) were fed *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) in a 30% w/v sucrose and distilled water suspension at a rate of $6 \times 10^3$ CFU/larva in accordance with USEPA OCSPP Microbial Pesticide Test Guideline 885.4380, *Honeybee Testing, Tier I*. Other groups (80 larvae/group) were similarly treated with 500 ppm potassium arsenate in 30% sucrose and 30% sucrose alone, and served as positive and negative controls, respectively. Seven days after treatment, the treated frames were removed from the hives and observed for larval removal or capping. The majority of larval mortality was observed at this observation period. Mortality in larvae treated with 30% sucrose only, *B. subtilis* var. *amyloliquefaciens* strain FZB24 in 30% sucrose, 500 ppm potassium arsenate in 30% sucrose was 6.25%, 7.5%, and 60.0%, respectively. Adult honeybee emergence began thirteen days after treatment and continued until Day 20. At study termination, one bee in each of the groups treated with 500 ppm potassium arsenate in 30% sucrose and 30% sucrose only were found capped and fully formed in the cell but dead. All other bees emerged from their cells as adults. Mortality was not significantly different between the groups treated with *B. subtilis* var. *amyloliquefaciens* strain FZB24 in 30% sucrose and 30% sucrose only. There were no toxic symptoms observed in the test group during the test period. This toxicity and pathogenicity study is classified as acceptable and satisfies the guideline requirement for a terrestrial arthropod study.

The acute toxicity of *B. subtilis* var. *amyloliquefaciens* strain FZB24 in earthworms (*Eisenia fetida*) was assessed over 14 days in accordance with Organization for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals No. 207, *Earthworm, Acute Toxicity Test*. Earthworms were exposed to five concentrations of *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient); 130, 216, 360, 600 and 1000 mg/kg artificial soil on a dry weight basis (equivalent to $7.8 \times 10^{10}$, $1.3 \times 10^{11}$, $2.2 \times 10^{11}$, $3.6 \times 10^{11}$ and $6 \times 10^{11}$ CFU/kg artificial soil). A negative control group and attenuated control group (1000 mg/kg artificial soil) were tested concurrently. There were no treatment related effects observed. The 14-day acute oral $LC_{50}$ was greater than 1000 mg/kg artificial soil or greater than $6 \times 10^{11}$ CFU/kg artificial soil and the no observed effect concentration (NOEC) was 1000 mg/kg artificial soil or $6 \times 10^{11}$ CFU/kg artificial soil. This earthworm study is classified as acceptable as a toxicity only study.

Requests to waive avian pulmonary, wild mammal and terrestrial plant testing were submitted by the applicant based on the rationale that no overt adverse effects were noted in acute mammalian studies and no report of plant pathogenicity by *B. subtilis* or *B. subtilis* var. *amyloliquefaciens* strain FZB24 was found by the USEPA following a search of published literature in the databases of Agricola, Current Contents and Science Direct. Similar literature searches conducted by the PMRA using the keywords "bacillus amyloquefaciens" and "bacillus subtilis" found numerous reports of antifungal/antimicrobial activities, single reports of mosquitocidal and nematicidal activities as well as reported infections in mammals, i.e. bovine mastitis, and some reproductive disorders in goats. With the exception of adverse effects to microorganisms, reports of adverse effects are very rare considering the ubiquitous nature of the microorganism. *Bacillus subtilis* is not considered as a mammalian pathogen and, from the data submitted under the Section 4.0, it was determined that *B. subtilis* var. *amyloliquefaciens* strain FZB (technical grade active ingredient) was not toxic or pathogenic to mammals via the oral, pulmonary, intravenous or dermal routes. Although the MPCA could adversely affect non-target microorganisms, the organism is not expected to affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes since it is a normal component of soil and plants.

Based on all the available data and information on the effects of *B. subtilis* var. *amyloliquefaciens* strain FZB24 to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods, terrestrial non-arthropod invertebrates, terrestrial plants, and microorganisms from the proposed use of Taegro.

### 4.2.2 Effects on Aquatic Organisms

Several studies were submitted to address the hazards of the MPCA to aquatic non-target organisms. These studies included non-target fish species, aquatic arthropods and algae.

In a 30-Day toxicity/pathogenicity study, juvenile rainbow trout (*Oncorhyncus mykiss*; ~70 days old; 10 fish/group) were exposed to *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) under static/renewal conditions in accordance with USEPA OCSPP Microbial Pesticide Test Guideline 885.4200, *Freshwater Fish Testing, Tier I*. Test fish were exposed to a geometric series of five test concentrations of technical grade active ingredient, an attenuated (heat inactivated) control and a negative (untreated) control. Nominal concentrations were $1.16 \times 10^9$, $2.31 \times 10^9$, $4.63 \times 10^9$, $9.25 \times 10^9$ and $1.85 \times 10^{10}$ CFU/L. Fish in technical grade active ingredient treatment group were also fed a diet containing $1.85 \times 10^9$ CFU *B. subtilis* var. *amyloliquefaciens* strain FZB24/kg. The concentration of attenuated *B. subtilis* var. *amyloliquefaciens* strain FZB24 in the attenuated control group was equivalent to that of the highest technical grade active ingredient treatment group, i.e. $1.85 \times 10^{10}$ CFU/L. One fish in the attenuated control died on Day 21. There were no mortalities in the negative control group or in the technical grade active ingredient treated group. There were also no signs of toxicity or other treatment-related effects observed during the study or at necropsy. The 30-day $LC_{50}$ for rainbow trout was greater than $1.85 \times 10^{10}$ CFU/L and the NOEC was $1.85 \times 10^{10}$ CFU/L. This toxicity and pathogenicity study is classified as acceptable and satisfies the guideline requirement for a freshwater fish study.

In a 21-day toxicity/pathogenicity study, daphnids (*Daphnia magna*; <24 hours old; 20/group) were exposed to *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) under static renewal conditions in accordance with the USEPA OCSPP Microbial Pesticide Test Guideline 885.4240–*Freshwater aquatic invertebrate testing, Tier I*. Daphnids were exposed to a geometric series of five test concentrations of technical grade active ingredient, an attenuated (heat inactivated) control and a negative (untreated) control. Nominal concentrations were $1.16 \times 10^9$, $2.31 \times 10^9$, $4.63 \times 10^9$, $9.25 \times 10^9$ and $1.85 \times 10^{10}$ CFU/L. The concentration of attenuated *B. subtilis* var. *amyloliquefaciens* strain FZB24 in the attenuated control group was equivalent to that of the highest technical grade active ingredient treatment group, i.e. $1.85 \times 10^{10}$ CFU/L. After 21 days, mortality in the negative and attenuated control groups was 5% and 0%, respectively, and all surviving daphnids appeared normal and healthy. Mortality in the $1.16 \times 10^9$, $2.31 \times 10^9$, $4.63 \times 10^9$, $9.25 \times 10^9$ and $1.85 \times 10^{10}$ CFU/L treatment groups was 5%, 0%, 0%, 0% and 0%, respectively. Adult daphnids in the negative and attenuated control group produced an average of 5.99 and 6.03 young per reproductive day respectively. Daphnids in the $1.16 \times 10^9$, $2.31 \times 10^9$, $4.63 \times 10^9$, $9.25 \times 10^9$ and $1.85 \times 10^{10}$ CFU/L treatment groups produced an average of 6.76, 6.39, 5.72, 4.18, and 1.33 young per reproductive day, respectively. Statistical analysis showed that reproduction was significantly reduced in groups treated with $9.25 \times 10^9$ and $1.85 \times 10^{10}$ CFU/L in comparison to the negative control group. The 21-day LOEC and NOEC values for reproduction were determined to be $9.25 \times 10^9$ and $4.63 \times 10^9$ CFU/L, respectively. The 21-day $LC_{50}$ for *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) was greater than $1.85 \times 10^{10}$ CFU/L. The screening level risk assessment was conducted based on the EEC for the highest use rate scenario for *B. subtilis* var. *amyloliquefaciens* strain FZB24 in Taegro on terrestrial crops (364 g Taegro /ha or $3.64 \times 10^{12}$ CFU/ha). The level of concern was not exceeded for daphnids. This toxicity and pathogenicity study is classified as acceptable and satisfies the guideline requirement for a toxicity/pathogenicity study for aquatic arthropods.

In a 72-hour toxicity study, the effect of *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) on the growth of the alga, *Scenedesmus subspicatus,* was studied in accordance with OECD Guideline for Testing of Chemicals No. 201, *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*. Exponentially growing cultures of algae were exposed to nominal concentrations of *B. subtilis* var. *amyloliquefaciens* strain FZB24 at $1 \times 10^{10}$, $5 \times 10^9$, $1 \times 10^9$, and $1 \times 10^8$ CFU/L (serial dilution factor of 1.73) and cell-free extracts of the technical grade active ingredient at rates equivalent to $1 \times 10^{10}$ and $1 \times 10^8$ CFU/L. The cell-free extracts were prepared by rapidly thawing a frozen preparation of spores. The algae at the different test concentrations did not show any abnormalities in comparison to control cultures. However, algal growth was inhibited at concentrations of $1 \times 10^8$ CFU/L and above. There were no such growth inhibition effects noted for cell-free extracts of *B. subtilis* var. *amyloliquefaciens* strain FZB24. The growth inhibition effect observed in technical grade active ingredient is not likely due to toxicity but rather a combination of indirect effects, namely decreasing availability of light due to increasing opacity in test suspensions as a result of large concentrations of spores and competition of nutrients between algae and growing spores in the nutrient media. Such effects are expected for suspensions with live microorganisms. This study is classified as acceptable, but is of limited utility for risk assessment purposes. No replacement study is required.

No toxicity/pathogenicity data were considered to address the potential for harm to aquatic non-target non-arthropod invertebrates and aquatic plants. As previously stated, literature searches conducted by the PMRA using the keywords "bacillus amyloquefaciens" and "bacillus subtilis" found no reports of adverse effects to aquatic non-arthropod invertebrates and aquatic plants. *Bacillus subtilis* is not considered as a non-arthropod invertebrate or plant pathogen. No further data are required to assess the risk of harm to aquatic non-target organisms.

Based on all the available data and information on the effects of *B. subtilis* var. *amyloliquefaciens* strain FZB24 to non-target aquatic organisms, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods, aquatic non-arthropod invertebrates or aquatic plants from the proposed use of Taegro. In addition, standard label statements will prohibit handlers from contaminating aquatic habitats during application, clean-up and repair.

### 4.3 Incident Reports related to the Environment

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website http://www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/incident/indexeng.php. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; American causality of highly probable, probable and possible) are considered in the reviews.

As of 25 September 2012, there were no environmental incidents reported in the PMRA Incident reporting database or in the USEPA's Ecological Incident Information System (EIIS) for products containing *B. subtilis* for use as pesticides.

## 5.0 Value

### 5.1 Effectiveness Against Pests

#### 5.1.1 Acceptable Efficacy Claims

##### 5.1.1.1 Partial suppression of fusarium wilt on greenhouse cyclamen

Four greenhouse trials on cyclamen were submitted in support of the proposed claim. Taegro was applied as a drench four to seven times at 0.2 g/L. Taegro applications did increase the number of marketable plants and flowers per plant, but these results were generally combined with low reduction in disease severity and plant mortality. Based on the submitted value information, the use of Taegro is supported for partial suppression of fusarium wilt on greenhouse cyclamen.

#### 5.1.1.2 Partial suppression of fusarium head blight on wheat

Twelve field trials were conducted across four U.S. States (Nebraska, North Dakota, Michigan, and Missouri) in 2009 and 2010. Certain trials were inoculated with *Fusarium graminearum*-infested corn kernels. Taegro was applied once at early flowering (245 g/ha). Treatments also included the commercial standard Prosaro 421 SC Foliar Fungicide.

Taegro reduced disease incidence and severity by an average of 7% and 15%, respectively, under moderate disease pressure and Taegro reduced Deoxynivalenol (DON) production (average of 12% reduction). According to crop experts, relatively low levels of protection may be sufficient to improve wheat grade and yield, and commercial standards rarely reduce DON levels in excess of 50% in research trials, with a great deal of variability between years.

Based on the efficacy of Taegro in comparison with Prosaro 421 SC Foliar Fungicide, the non-conventional status of the product as well as the lack of registered fungicides to manage fusarium head blight in organic wheat production, the use of Taegro is supported for partial suppression of fusarium head blight on wheat. Taegro must be used in conjunction with integrated pest management (IPM) practices in order to adequately manage mycotoxin levels in wheat kernels.

#### 5.1.1.3 Suppression of bottom rot on greenhouse and field lettuce

Three field trials from California were conducted on early-season head lettuce under natural inoculation conditions. Taegro was sprayed up to seven times at 7-day intervals to the plant base and soil. Four application rates were tested (175, 182, 364 and 728 g/ha) and generally provided statistically comparable disease reduction. Taegro rates close to the proposed rate of 190 g/ha significantly reduced bottom rot incidence and severity in two out of three trials. Levels of protection reached up to 53% under severe disease pressure. Results from one trial show that additional applications of Taegro substantially improved bottom rot management. Based on this value information, the use of Taegro is supported for suppression of bottom rot on greenhouse and field lettuce. Extrapolation from field to greenhouse was considered acceptable based on the controlled environmental conditions occurring in the latter.

#### 5.1.1.4 Suppression of late blight on greenhouse and field tomato

One field trial from California was conducted on transplanted tomatoes under natural disease pressure from late blight. Taegro was applied as a foliar spray at 182, 273 or 364 g/ha. Eight applications were made on a 7-day spray schedule, starting 30 days after planting. Late blight symptoms were first observed in the untreated control on October 1st, 2009 (7 days after application). Three weeks later, 100% disease incidence and 72% disease severity was noted in the untreated control.

The tested fungicides did not provide consistent reduction of late blight incidence under increasing disease pressure. Among the three Taegro rates, only the higher rate of 364 g/ha showed significantly lower disease incidence than the untreated control, and at certain assessment dates only. This rate was also the only one that adequately suppressed late blight severity (56% reduction), a result that is statistically similar to that achieved with the commercial

standard Tanos 50DF Fungicide. Furthermore, treatment with Taegro at 364 g/ha numerically increased the number and weight of harvested tomatoes.

Based on this value information, the use of Taegro is supported for suppression of tomato late blight, when applied at 364 g/ha. Extrapolation from field to greenhouse was considered acceptable based on the controlled environmental conditions occurring in the latter.

### 5.2 Economics

No economic analysis was performed for this submission.

### 5.3 Sustainability

#### 5.3.1 Survey of Alternatives

Refer to Appendix I, Table 3 for a summary of the active ingredients currently registered for the uses supported with Taegro.

#### 5.3.2 Compatibility with Current Management Practices Including Integrated Pest Management

The compatibility of Taegro with conventional fungicides has not been extensively tested and no claims are made in that regard. Otherwise, when used as recommended, Taegro would not interfere with the cultural and sanitation practices intended to prevent disease development in crops. For management of fusarium head blight in wheat, Taegro must be applied in conjunction with IPM practices to adequately reduce mycotoxin levels in kernels.

#### 5.3.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

*B. subtilis* var. *amyloliquefaciens* strain FZB24, the active ingredient in Taegro, is a non-conventional microbial fungicide with a multi-site mode of action. Consequently, development of disease resistance to the fungicidal activity of this bacterium is not expected.

#### 5.3.4 Contribution to Risk Reduction and Sustainability

Taegro is the first non-conventional fungicide registered to manage fusarium head blight on wheat and bottom rot on greenhouse and field lettuce. Its registration would be especially valuable in organic wheat production, which is becoming more prevalent in Canada due to consumer demand, and where no conventional products may be applied.

# 6.0 Toxic Substances Management Policy Considerations

## 6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy, which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

While reviewing Taegro Technical, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in the technical product, Taegro Technical, and formulants in the end-use product, Taegro. The PMRA has reached the following conclusions:

Taegro Technical does not meet the Track 1 criteria because the active ingredients is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track 1 criteria.

Therefore, the use of Tagero is not expected to result in the entry of Track 1 substances into the environment.

## 6.2 Formulants and Contaminants of Health or Environmental Concern

Taegro Technical does not contain any formulants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* (Amended June 25, 2008 SI\2008-67). There are also no formulants or contaminants of health or environmental concern present in the associated end-use product, Taegro.

# 7.0 Summary

## 7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for Taegro Technical and Taegro were deemed adequate to assess their potential human health and environmental risks. The technical grade active ingredients were characterized and the specifications of the end-use product were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of 1 year when stored at 4–35°C.

## 7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *B. subtilis* var. *amyloliquefaciens* strain FZB24 were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests, spores of *B. subtilis* var. *amyloliquefaciens* strain FZB24 were of low toxicity by the oral, pulmonary and dermal routes, and they were not pathogenic or infective via the oral, intravenous injection and pulmonary exposure routes in animals. The technical grade active ingredient and the end-use product have the potential to irritate eyes and skin, and they are considered to be potential sensitizers.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation.

Label statements (i.e. Potential Sensitizer, may cause sensitization) and risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, goggles, NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal.

## 7.3 Environmental Risk

The non-target organism testing, scientific rationales and supporting published scientific literature submitted in support of Tagero Technical and Tagero (containing *B. subtilis* var. *amyloliquefaciens* strain FZB24) were determined to be sufficiently complete. The use of Taegro containing *B. subtilis* var. *amyloliquefaciens* strain FZB24 is not expected to pose a risk to birds, mammals, arthropods, fish, and plants when the directions for use on the label are followed. No other environmental fate studies or non-target organism studies are required to assess the risk of Taegro used as a commercial-class biological fungicide to suppress bottom rot on lettuce (greenhouse and field), late blight on tomato (greenhouse and field), *Fusarium* wilt on greenhouse cyclamen and *Fusarium* head blight on wheat.

As a specific precaution, the Taegro label instructs users to not contaminate irrigation or drinking water supplies or aquatic habitats by application of product, cleaning of equipment or disposal of wastes.

### 7.4 Value

The value information data submitted to register Taegro is adequate to support the following claims:

- partial suppression of fusarium wilt on greenhouse cyclamen
- partial suppression of fusarium head blight on wheat
- suppression of bottom rot on greenhouse and field lettuce
- suppression of late blight on greenhouse and field tomato

## 8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of Taegro Technical and Taegro, containing the technical grade active ingredient *Bacillus subtilis* var. *amyloliquefaciens* Strain FZB24, to suppress fusarium wilt on greenhouse cyclamen, fusarium head blight on wheat; bottom rot on lettuce and late blight on tomato.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

## List of Abbreviations

| | |
|---|---|
| °C | degree(s) Celsius |
| a.i. | active ingredient |
| ADI | acceptable daily intake |
| ARfD | acute reference dose |
| bw | body weight |
| CDPR | California Department of Pesticide Regulation |
| CFU | colony forming unit |
| cm | centimetres |
| DON | Deoxynivalenol |
| DSMZ | German Collection of Microoganisms and Cell Cultures |
| EEC | expected environmental concentration |
| EIIS | Ecological Incident Information System |
| g | gram |
| ha | hectare(s) |
| HE | high efficiency |
| IPM | integrated pest management |
| kg | kilogram |
| L | litre |
| $LC_{50}$ | lethal concentration 50% |
| $LD_{50}$ | lethal dose 50% |
| LOC | level of concern |
| LOEC | low observed effect concentration |
| MCC | maximum challenge concentration |
| mg | milligram |
| mL | millilitre |
| MPCA | microbial pest control agent |
| MRL | maximum residue limit |
| N/A | not applicable |
| NC | not classified |
| NIOSH | National Institute for Occupational Safety and Health |
| NOEC | no observed effect concentration |
| NOEL | no observed effect level |
| OCSPP | Office of Chemical Safety and Pollution Prevention |
| OECD | Organization for Economic Cooperation and Development |
| PCR | Polymerase Chain Reaction |
| PMRA | Pest Management Regulatory Agency |
| PPE | personal protective equipment |
| ppm | parts per million |
| rRNA | ribosomal ribonucleic acid |
| RQ | risk quotient |
| TG | treatment group |
| TSMP | Toxic Substances Management Policy |
| USEPA | United States Environmental Protection Agency |
| w/v | weight per volume |

# Appendix I Tables and Figures

## Table 1 Toxicity and Infectivity of Taegro Technical (*B. subtilis* var. *amyloliquefaciens* strain FZB24) and its associated End-use Product, Taegro

| Study Type | Species, Strain, and Doses | Results | Significant Effects and Comments | Reference(s) |
|---|---|---|---|---|
| **Acute Toxicity/Infectivity of *B. subtilis* var. *amyloliquefaciens* strain FZB24 (Taegro Technical)** | | | | |
| Acute Oral Toxicity and Infectivity | Rat- CD, 15/sex, 1 mL suspension in sterile purified water ($1.3 \times 10^8$ CFU/animal), interim sacrifices on Days 0, 3, 7, and 14<br><br>15/sex, 1 mL heat-killed test substance ($1.3 \times 10^8$ CFU/animal) suspension in sterile purified water | $LD_{50} > 1.3 \times 10^8$ CFU/animal | There were no mortalities or treatment-related effects on body weight/bodyweight gain or relative organ/tissues weights.<br><br>Rough hair coat was observed for one animal on Day 1.<br><br>No abnormal necropsy findings.<br><br>The MPCA was cleared from all tissues by Day 7.<br><br>**LOW TOXICITY AND NO PATHOGENICITY**<br><br>**ACCEPTABLE** | PMRA 2122466 |
| Acute Pulmonary Toxicity and Infectivity | Rat-CD<br><br>Treatment group (TG): 15/sex, 0.1 mL suspension in sterile purified water ($1.4 \times 10^8$ CFU/animal), interim sacrifices on Days 0, 7, 21, and 35<br><br>15/sex, 0.1 mL heat-killed test substance ($1.4 \times 10^8$ CFU/animal) suspension in sterile purified water | $LD_{50} > 1.4 \times 10^8$ CFU/animal | There were no mortalities or treatment-related clinical signs of toxicity.<br><br>No treatment related effects on body weight/bodyweight gain.<br><br>Female (treatment group) animals had significantly increased relative lung and associated lymph nodes weights on Day 7 compared to naïve control group (normal immunological reaction to foreign material).<br><br>Total clearance of the test substance from the lungs of treated animals was not achieved.<br><br>**LOW TOXICITY AND NO PATHOGENICITY**<br><br>**ACCEPTABLE** | PMRA 2122468 |

| Study Type | Species, Strain, and Doses | Results | Significant Effects and Comments | Reference(s) |
|---|---|---|---|---|
| Acute Intravenous Injection | Rat – CD<br><br>Treatment group (TG): 12/sex, 0.5 mL suspension in sterile purified water ($1.0 \times 10^7$ CFU/animal), interim sacrifices on Days 0, 7, 21, and 35<br><br>12/sex, treated with autoclaved 0.5mL suspension in sterile purified water ($1.0 \times 10^7$ spores) | Not pathogenic<br><br>$LD_{50} > 1.0 \times 10^7$ CFU/animal | There were no mortalities or treatment-related clinical signs of toxicity.<br><br>No treatment related effects on body weight/bodyweight gain.<br><br>Relative organ/tissue weights showed no treatment-related differences.<br><br>No abnormal findings at necropsy.<br><br>Test substance was cleared by Day 35 from all tissues except for detection in the spleen of female treatment group animals.<br><br>**NOT PATHOGENIC**<br>**ACCEPTABLE** | PMRA 2122470 |
| Acute Dermal Toxicity and Irritation<br><br>Irritation was scored by the method of Draize. | Rabbit- New Zealand white, 5/sex, 2000 mg/kg bw ($1.5–2 \times 10^{12}$ CFU/animal) administered to 10% of the body surface and exposure period of 24 h | $LD_{50} > 2000$ mg/kg bw | No mortality or signs of treatment-related systemic effects.<br><br>All animals exhibited edema, erythema and eschar formation at the application sites during the study. Edema (9 animals) and erythema (10 animals) were initially seen within 1.5 h after removal of the dressings.<br><br>Irritation was cleared by Day 11.<br><br>**LOW TOXICITY and SEVERELY IRRITATING**<br><br>**ACCEPTABLE** | PMRA 2122475 |

| Study Type | Species, Strain, and Doses | Results | Significant Effects and Comments | Reference(s) |
|---|---|---|---|---|
| Dermal Irritation<br><br>Irritation was scored by the method of Draize. | Rabbit- New Zealand white, 3 females were exposed dermally to a dose of 0.5 g of the granular test substance ($7.0 \times 10^{10}$ CFU/g) and a single dose of 0.5 mL of 1.5% suspension in water for 4 h under occlusion. | Dermal irritation score = 0 | No signs of irritation during the 72-h observation period.<br><br>**NON-IRRITATING**<br><br>**ACCEPTABLE** | PMRA 2122477 |
| **Acute Toxicity/Irritation of Taegro** | | | | |
| Acute Dermal Toxicity and Irritation – end-use product<br><br>Irritation was scored by the method of Draize | Rabbit- New Zealand white, 5/sex were dermally exposed to end-use product at a dose of 2000 mg/kg bw ($1.7–2.3 \times 10^{11}$ CFU/animal) for 24 h under occlusion to an area of approximately 10% bw | $LD_{50}$ > 2000 mg/kg bw | No mortality or signs of treatment-related systemic effects.<br><br>All animals gained weight.<br><br>All animals exhibited erythema, eight animals exhibited edema, and six animals exhibited eschar formation at the application sites during the study. Edema and erythema were first seen within 1.5 h following the removal of wrappings. Five animals showed severe erythema on Day 3.<br><br>Animals were free of dermal irritation by Day 8.<br><br>**LOW TOXICITY and SEVERELY IRRITATING**<br><br>**ACCEPTABLE** | PMRA 2122526 |

## Table 2 Toxicity to Non-Target Species

| Organism | Exposure | Protocol | Significant Effect, Comments | Reference |
|---|---|---|---|---|
| **Terrestrial Organisms** | | | | |
| **Vertebrates** | | | | |
| Birds | *Colinus virginianus* (21 days old)<br><br>30-day oral | Birds (30) were gavaged with *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) at a dose of 10 mL/kg bw or $1\times10^9$ CFU/g bw for 5 consecutive days.<br><br>Attenuated control group (10 birds) was gavaged with *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) at a dose of 10 mL/kg bw for 5 consecutive days.<br><br>Negative control group (10 birds) was dosed with reverse osmosis water at a dose of 10 mL/kg bw for 5 consecutive days.<br><br>Birds were observed for 30 days. | No treatment related mortalities or overt signs of toxicity were reported in the treatment groups.<br><br>There were no signs of pathogenicity or infectivity.<br><br>30-day acute oral $LD_{50} > 1\times10^9$ CFU/g bw for 5 consecutive days.<br><br>30-day NOEL $1\times10^9$ CFU/g bw for 5 consecutive days.<br><br>**ACCEPTABLE** | PMRA 2122480 |
| | Pulmonary | A request to waive the requirement for test data was submitted based on the scientific rationale that no toxicity or pathogenicity was observed in the avian oral study or the acute mammalian studies (see Section 3.1). Avian pulmonary testing was waived. | | PMRA 2122481 |
| Wild Mammals | A request to waive the requirement for test data was submitted based on the scientific rationale that no toxicity or pathogenicity was observed the acute mammalian studies submitted for human health a safety testing (see Section 3.1). *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) was not toxic to mammals via the oral, pulmonary or dermal routes, they were also not pathogenic via the oral, pulmonary or intravenous route. No further data are required to assess the risk of harm to non-target wild mammals. | | | PMRA 2122482 |

| Organism | Exposure | Protocol | Significant Effect, Comments | Reference |
|---|---|---|---|---|
| **Invertebrates** | | | | |
| **Arthropods** | | | | |
| Terrestrial Arthropods | *Apis mellifera* (adult bees)<br><br>29-day dietary | Four replicates (175 bees) were fed daily with *B. subtilis* var. *amyloliquefciens* strain FZB24 (technical grade active ingredient) in a 30% w/v sucrose/water suspension at a concentration of $10^5$ CFU/mL for 29 days.<br><br>One negative control (30% w/v sucrose only) with four replicates of bees (170 bees). | Study was terminated on Day 29 after all the bees died.<br><br>Test group mortality was not significantly different from negative control throughout the study.<br><br>There were signs of toxicity or pathogenicity.<br><br>The 29-day $LC_{50}$ was greater than $1\times10^6$ CFU/mL.<br><br>**ACCEPTABLE** | PMRA 2122485 |
| | *Apis mellifera* (larvae; 3 days old)<br><br>20-day dietary | Four replicates of 20 were fed with *B. subtilis* var. *amyloliquefciens* strain FZB24 (technical grade active ingredient) in a 30% w/v sucrose/water suspension at a dose of $6\times10^3$ CFU/larva.<br><br>Four replicates of 20 were fed 500 ppm potassium arsenate in a 30% w/v sucrose/water suspension (positive control).<br><br>Four replicates of 20 were fed 30% sucrose/water suspension only (negative control)<br><br>Observed for mortality and adult emergence until Day 20. | Adult emergence began on Day 13 in all treatment groups and continued until Day 19.<br><br>Two cells remained capped on Day 20 in negative and positive control groups. One bee was found in each cell fully formed but dead.<br><br>Larval mortality (empty cells prior to emergence) was highest in positive control, i.e. 61.25% on Day 7.<br><br>Larval mortality in negative control and technical grade active ingredient-treated groups was 7.5% on Day 7.<br><br>No unusual behaviours were noted for any of the emerged adult bees.<br><br>$LC_{50}$ was greater than $6\times10^3$ CFU/larva.<br><br>**ACCEPTABLE** | PMRA 2122486 |

| Organism | Exposure | Protocol | Significant Effect, Comments | Reference |
|---|---|---|---|---|
| **Non-arthropods** | | | | |
| Terrestrial Non-Arthropod Invertebrates | *Eisenia fetida* (adult with clitellum)<br><br>14-day exposure | Four replicates of 10 were exposed to *B. subtilis* var. *amyloliquefciens* strain FZB24 (technical grade active ingredient) in artificial soil at concentrations of $8\times10^{10}$, $1\times10^{11}$, $2\times10^{11}$, $4\times10^{11}$, $6\times10^{11}$ CFU/kg soil.<br><br>Four replicates of 10 were exposed to inactivated *B. subtilis* var. *amyloliquefciens* strain FZB24 (Attenuated Control) in artificial soil at a concentration that is equivalent to $6\times10^{11}$ CFU/kg soil.<br><br>Four replicates of 10 remained untreated (negative control).<br><br>On Days 7 and 14, earthworms were placed on clean paper and counted. Earthworms were also observed for signs of toxicity. | There were no mortalities in any of the groups.<br><br>All earthworms were normal in appearance and behaviour.<br><br>14-day $LC_{50}$ was greater than $6\times10^{11}$ CFU/kg soil.<br><br>NOEC was $6\times10^{11}$ CFU/kg soil.<br><br>**ACCEPTABLE** | PMRA 2122488 |
| **Plants** | | | | |
| Plants | A request to waive the requirement for test data was submitted based on the scientific rationale that no reports of adverse effects were found following a search of published scientific literature. No further data are required to assess the risk of harm to non-target terrestrial plants. | | | PMRA 2122489 |
| **Microorganisms** | | | | |
| Micro-organisms | A request to waive the requirement for test data was not submitted. The MPCA is not expected to affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes since it is a normal component of soil and plants. No further data are required to assess the risk of harm to non-target microorganisms. | | | |

| Organism | Exposure | Protocol | Significant Effect, Comments | Reference |
|---|---|---|---|---|
| **Aquatic Organisms** | | | | |
| | | **Vertebrates** | | |
| Fish | *Oncorhynchus mykiss* (juveniles; ~70 days old)<br><br>30-day static renewal | Groups of 10 fish were exposed to $1.16\times10^9$, $2.31\times10^9$, $4.63\times10^9$, $9.25\times10^9$ and $1.85\times10^{10}$ CFU *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient)/L. Fish were also fed a diet containing $1.85\times10^9$ CFU *B. subtilis* var. *amyloliquefaciens* strain FZB24/kg<br><br>A group of 10 fish was exposed to inactivated *B. subtilis* var. *amyloliquefaciens* strain FZB24 (Attenuated Control) at a concentration equivalent to $1.85\times10^{10}$ CFU/L.<br><br>One negative control group (10 fish) held in untreated test water.<br><br>Daily observations for mortality and signs of toxicity/pathogenicity. | One fish (1/10) in the attenuated control group was found dead on Day 21. There were no other mortalities in any of the groups.<br><br>All the fish appeared normal throughout the study.<br><br>A gross necropsy was performed on three fish from each treatment group. No signs of toxicity or infectivity were found.<br><br>The 30-day $LC_{50}$ for rainbow trout was greater than $1.85\times10^{10}$ CFU/L.<br><br>The NOEC was $1.85\times10^{10}$ CFU/L.<br><br>**ACCEPTABLE** | PMRA 2122484 |
| | | **Invertebrates** | | |
| Aquatic Arthropods | *Daphnia magna*<br><br>21-day static renewal | Four replicates of 5 daphnids were exposed to $1.16\times10^9$, $2.31\times10^9$, $4.63\times10^9$, $9.25\times10^9$ and $1.85\times10^{10}$ CFU *B. subtilis* var. *amyloliquefaciens* strain FZB24/L.<br><br>Four replicates of 5 daphnids were exposed to inactivated *B. subtilis* var. *amyloliquefaciens* strain FZB24 (Attenuated Control) at a concentration equivalent to | One daphnid (1/20) in negative control group and in the treatment group exposed to $1.16\times10^9$ CFU/L.<br><br>All surviving daphnids appeared normal and healthy.<br><br>Adult daphnids in the negative and attenuated control group produced an average of 5.99 and 6.03 young per reproductive day respectively.<br><br>Daphnids in the $1.16\times10^9$, $2.31\times10^9$, $4.63\times10^9$, $9.25\times10^9$ and $1.85\times10^{10}$ CFU/L treatment groups produced an average of | PMRA 2122487 |

| Organism | Exposure | Protocol | Significant Effect, Comments | Reference |
|---|---|---|---|---|
| | | $1.85\times10^{10}$ CFU/L.<br><br>Negative control with four replicates of 5 daphnids in untreated test culture.<br><br>Observed for mortality, reproduction, and body length for 21 days. | 6.76, 6.39, 5.72, 4.18, and 1.33 young per reproductive day, respectively.<br><br>21-day LOEC and NOEC values for reproduction were determined to be $9.25\times10^{9}$ and $4.63\times10^{9}$ CFU/L, respectively.<br><br>21-day $LC_{50}$ was greater than $1.85\times10^{10}$ CFU/L.<br><br>**ACCEPTABLE** | |
| Aquatic Non-Arthropod Invertebrates | A request to waive the requirement for test data was not submitted. However, no reports of adverse effects to aquatic non-arthropod invertebrates were found in searches of published scientific literature. No further data are required to assess the risk of harm to non-target aquatic non-arthropod invertebrates. | | | |
| **Plants** | | | | |
| Aquatic Plants | *Scenedesmus subspicatus*<br><br>72-hour static | Algal cultures were exposed to *B. subtilis* var. *amyloliquefaciens* strain FZB24 (technical grade active ingredient) at concentrations of $1\times10^{10}$, $5\times10^{9}$, $1\times10^{9}$, and $1\times10^{8}$ CFU/L (serial dilution factor of 1.73).<br><br>Cell-free control daphnids were exposed to cell-free extracts of the technical grade active ingredient at rates equivalent to $1\times10^{10}$ and $1\times10^{8}$ CFU/L.<br><br>Negative control daphnids were untreated.<br><br>*The cell-free extracts were prepared by rapidly thawing a frozen spore preparation (-80°C). | None of the daphnids showed any abnormalities in comparison to negative control cultures.<br><br>Algal growth was inhibited at concentrations of $1\times10^{8}$ CFU/L and above.<br><br>No growth inhibition was observed for cell-free extracts of *B. subtilis* var. *amyloliquefaciens* strain FZB24.<br><br>**ACCEPTABLE BUT IS OF LIMITED UTILITY FOR RISK ASSESSMENT PURPOSES** | PMRA 2122490 |

**Table 3 Summary of Fungicide Alternatives for the Uses Supported with Taegro**

| Crop | Pests | Active Ingredient and Resistance Management Group |
|---|---|---|
| greenhouse cyclamen | fusarium wilt | - thiophanate-methyl (1)<br>- *Streptomyces griseoviridis* strain K61 (NC)<br>- *Trichoderma asperellum* strain T34 (NC) |
| wheat | fusarium head blight | - metconazole (3)<br>- metconazole (3) + pyraclostrobin (11)<br>- prothioconazole (3)<br>- prothioconazole (3) + tebuconazole (3)<br>- tebuconazole (3)<br>- chlorothalonil (M5) |
| greenhouse and field lettuce | bottom rot | N/A |
| greenhouse and field tomato | late blight | ***Greenhouse***<br>- mandipropamid (40)<br>- copper oxychloride (M1)<br>- mancozeb (M3)<br>- chlorothalonil (M5)<br>- garlic (NC) |
| | | ***Field***<br>- fluoxastrobin (11)<br>- propamocarb hydrochloride (28)<br>- mono- and di-potassium salts of phosphorous acid (33)<br>- mandipropamid (40)<br>- dimethomorph (40) + ametoctradin (45)<br>- fluopicolide (43)<br>- copper oxychloride (M1)<br>- copper sulphate (M1)<br>- mancozeb (M3)<br>- maneb (M3)<br>- metiram (M3)<br>- ziram (M3)<br>- captan (M4)<br>- chlorothalonil (M5) |

## Table 4 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

| Proposed claim | Supported / Unsupported |
|---|---|
| **Greenhouse cyclamen**: suppression of fusarium head blight (*Fusarium graminearum*) with foliar applications at 256 g/ha (7-day intervals) | Supported for partial suppression. |
| **Wheat**: suppression of fusarium wilt (*Fusarium oxysporum* f. sp. *cyclaminis*) with drench applications at 20 g/100 L water (7-day intervals). | Supported for partial suppression. |
| **Greenhouse and field lettuce**: suppression of bottom rot (*Rhizoctonia solani*) with applications made to the plant base and soil surface at 190 g/ha (7-day intervals). | Supported. |
| **Greenhouse and field tomato**: suppression of late blight (*Phytophthora infestans*) with foliar applications at 364 g/ha (7-day intervals). | Supported. |

# References

## A. List of Studies/Information Submitted by Registrant

### 1.0 Chemistry

| PMRA Document Number | Reference |
|---|---|
| 2122445 | Taegro Label, DACO: M1.1, CBI |
| 2122447 | 2011. Product Profile and Proposed Use Patterns. DACO: M1.2, M1.3 CBI |
| 2122509 | DACO: M1.1, CBI |
| 2122510 | Submission Information, DACO: M1.2,M1.3, CBI |

**Methods of Analysis**

| PMRA Document Number | Reference |
|---|---|
| 2122446 | Taegro Technical. DACO: M1.2, M2.7.1 CBI |
| 2122449 | Taegro_Technical_M2.1-M2.7.2. DACO: M2.1, M2.2, M2.3, M2.4, M2.5, M2.6, M2.7.1, M2.7.2 CBI |
| 2122450 | Use of *Bacillus subtilis* as a biocontrol agent. Salt stress tolerance induction by *Bacillus subtilis* FZB24 seed treatment in tropical vegetable field crops and its mode of action. DACO: M2.7.2 |
| 2122451 | Growth parameters of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 at various temperatures. DACO: M2.7.2 CBI |
| 2122452 | On the safety of *Bacillus subtilis* and *B. amyloloiquefaciens*: a review. DACO: M2.7.2 |
| 2122453 | 5 Batch Analysis for Taegro Technical. DACO: M2.10.1, M2.10.2, M2.8, M2.9.2, M2.9.3 CBI |
| 2122454 | Description of beginning materials and manufacturing process for *Bacillus subtilis* var *amyloliquefaciens* Strain FZB24. DACO: M2.8 CBI |
| 2122455 | 2011. Taegro_Technical_M2.9.1a. DACO: M2.9.1 CBI |
| 2122456 | MSDS for Taegro Technical. DACO: M2.9.1 |
| 2122458 | 2010. Product Specification. DACO: M2.9.1 CBI |
| 2122459 | Product Identification of Taegro Technical. DACO: M2.10.1 CBI |
| 2122460 | Storage Stability Study for Taegro Technical. DACO: M2.11, M2.12 CBI |
| 2122461 | Determination of Color, Physical State, Odor, pH, and Density of Taegro Technical. DACO: M2.12 CBI |
| 2122462 | pH and Bulk Density Determination of Taegro Technical. DACO: M2.12 CBI |
| 2122463 | Moisture Content Determination of Taegro Technical. DACO: M2.12 CBI |
| 2122464 | Determination of the Corrosion Characteristics of *Bacillus subtilis* var *amyloliquefaciens* End Use Product. DACO: M2.12 CBI |
| 2122511 | Submission Information, Taegro_EP. DACO: M2.1, M2.2, M2.3, M2.4, M2.5, M2.6, M2.7.1, M2.7.2 CBI |
| 2122512 | 5 Batch Analysis for Taegro. DACO: M2.10.1, M2.10.2, M2.8, M2.9.2, M2.9.3 CBI |

2122513 Description of Beginning Materials and Manufacturing Process for Taegro. DACO: M2.8 CBI
2122514 Statement of Product Specification. DACO: M2.9.1 CBI
2122515 Material Safety Data Sheet. DACO: M2.9.1
2122516 PMRA_Taegro_EP_M2.9.1c. DACO: M2.9.1
2122517 Material Safety Data Sheet. DACO: M2.9.1
2122518 Material Safety Data Sheet. DACO: M2.9.1
2122519 Product Identification of Taegro. DACO: M2.10.1
2122520 2011 Storage Stability Study for Taegro. DACO: M2.11, M2.12 CBI
2122521 1998 Determination of the Colour, Physical State, Odour, pH, and Density of Taegro. DACO: M2.12 CBI
2122522 2011, pH and Bulk Density Determination of Taegro. DACO: M2.12 CBI
2122523 2011, Moisture Content Determination of Taegro. DACO: M2.12 CBI
2122524 1999, Determination of the Corrosion Characteristics of Taegro. DACO: M2.12 CBI
2224163 DACO: M2.8 CBI


## 2.0 Human and Animal Health


| PMRA Document Number | Reference |
|---|---|
| 2122465 | Summary of Infectivity and Toxicity. DACO: M4.2.1. |
| 2122466 | Toxicity/Pathogenicity Testing of Taegro Technical following Oral Challenge in Rates. DACO: M4.2.2. |
| 2122468 | Toxicity/Pathogenicity Testing of Taegro Technical following Acute Intratracheal Challenge in Rates. DACO: M4.2.3. |
| 2122469 | Summary of Acute Infectivity. DACO: M4.3.1. |
| 2122470 | Toxicity/Pathogenicity of Taegro Technical Following Acute Intravenous Challenge in Rats. DACO: M4.3.2. |
| 2122475 | Acute Dermal Toxicity/Pathogenicity of Taegro Technical in Rabbits. DACO: M4.4. |
| 2122477 | Summary of Irritation. DACO: M4.5.1. |
| 2122492 | *Bacillus subtilis*: Final Registration review. DACO: M12.5.4, M12.5.9. |
| 2122526 | PMRA_Taegro_EP_M4.4. DACO: M4.4. |
| 2122525 | PMRA_Taegro_EP_M4.3.1. DACO: M4.3.1. |


## 3.0 Environment


| PMRA Document Number | Reference |
|---|---|
| 2122480 | Taegro Technical: An Avian Oral Pathogenicity and Toxicity Study in the Northern Bobwhite. DACO: M9.2.1. |
| 2122481 | Waiver Request for Avian Pulmonary Testing Requirements. DACO: M9.2.2. |
| 2122482 | Waiver request for Wild Animal Testing Requirements. DACO: M9.3. |
| 2122484 | Taegro Technical: A Five Concentration Toxicity and Pathogenicity Test with |

Rainbow trout. DACO: M9.4.1.
2122485 Evaluation of Dietary effects of Taegro Technical on Adult Honey Bees. DACO: M9.5.1.
2122486 Evaluation of Dietary effects of Taegro Technical on Honey Bee Larvae. DACO: M9.5.1.
2122487 Taegro Technical: A 21 Day Life Cycle Toxicity and Pathogenicity Test with the Cladoceran. DACO: M9.5.2.
2122488 Taegro Technical: An Acute Toxicity Study with the earthworm in an Artificial Soil Substrate. DACO: M9.6.
2122489 Waiver Request for Terrestrial Plant Testing Requirements. DACO: M9.8.1.
2122490 Algae Growth Inhibition Test - Test Article: Taegro Technical. DACO: M9.8.2.
2122491 DACO: M12.5.4, M12.5.9.
2122492 *Bacillus subtilis*: Final Registration review. DACO: M12.5.4, M12.5.9.

## 4.0 Value

| PMRA Document Number | Reference |
|---|---|
| 2122527 | Summary of Value M10.1, DACO: M10.1 |
| 2122528 | Efficacy Data for Taegro, DACO: M10.2.2 |
| 2122532 | Efficacy Data for Taegro, DACO: M10.2.2 |
| 2122533 | Efficacy Data for Taegro, DACO: M10.2.2 |
| 2122534 | Efficacy Data for Taegro, DACO: M10.2.2 |
| 2122535 | 2010 Uniform Biological Control Trials – Preliminary Results, DACO: M10.2.2 |
| 2122536 | Results of 2009 Uniform Biological Control Trials, DACO: M10.2.2 |
| 2122537 | Efficacy Data for Taegro, DACO: M10.2.2 |
| 2122538 | 2009, Efficacy of NZBPC1100 for control of *Rhizoctonia sol*ani in Lettuce, DACO: M10.2.2 |
| 2122539 | 2007, Efficacy of NZBPC1100 for control of *Rhizoctonia sol*ani in Lettuce, DACO: M10.2.2 |
| 2122540 | 2009, Efficacy of NZBPC1100 for control of *Phytophthora infestans* in Tomato, DACO: M10.2.2 |
| 2122541 | Efficacy Data for Taegro, DACO: M10.2.2 |
| 2122543 | 2009, Efficacy of NZBPC1100 for control of *Phytophthora capsici* in Pepper and Tomato, DACO: M10.2.2 |
| 2122544 | 2011, submission information, DACO: M10.3.1,M10.3.2.1,M10.3.2.2,M10.4.1,M10.4.2,M10.4.3,M10.4.4 |